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(54) STOPPER HAVING A CAVITY FOR REAGENTS AND AN ASSAY METHOD USING SAID STOPPER

STOPFEN MIT EINER KAMMER FÜR REAGENZIEN UND ANALYSE-METHODE FÜR DIE VERWENDUNG DIESES STOPFENS

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(56) References cited:
EP-A- 0 093 090
EP-A- 0 215 735
US-A- 4 192 429

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Description

[0001] The present invention relates to a closure device suitable for use in performing an assay, particularly a clinical test on a biological fluid such as blood. It is an object of the invention to provide a closure device which can render the reagent used in the assay into a state assuring a reliable test result and is able to maintain said state of the reagent over a long period of storage as well as under the stress of adverse environmental conditions, and furthermore so as to permit the addition of the reagent into the sample under assay at a desired instant of time.

[0002] It is a further object of the invention to provide a closure device permitting said assay to be carried out under maximally protected conditions thus on one hand eliminating the error factors imposed on the assay results by the environment and on the other hand reducing the contamination risk imposed by the assay on the environment.

[0003] These goals and others can be attained by virtue of the closure device according to the invention, said device having its basic construction designed into a closure assembly suitable for closing the diagnostic test vessel, into which closure device the reagent after its preparation into an advantageous state for the assay is sealed in a manner permitting the release of the reagent from the closure device into the diagnostic test vessel at a desired instant of time.

[0004] Closure devices having a similar basic construction are known in the art, and their use has been contemplated in, e.g., the preparation of pharmaceuticals. In this application, the effective therapeutic drug is prepared from its basic constituents not earlier than at its required instant of use, whereby one or a number of the constituents of the pharmaceutical product are added to the other constituents stored in medicine bottle or similar container just before the use of the drug. Prior to its addition, the first constituent(s) may have been stored in a closed space such as, e.g., the closure of the medicine bottle, wherefrom it can be taken into use by depressing or similarly actuating action on the closure, said action opening a passageway from the interior of the closure into the medicine bottle. Closure devices having this basic construction are described, i.a., in patent publications GB 1,193,989, GB 1,479,370, EP 0,093,090, EP 0,338,349, EP 0,561,322 and EP 0,344,849.

[0005] These conventional closures fail, however, to take into account the fact that a successful test in a great number of medical or similar applications requires the reagent used in the test to be in an advantageous state for the assay and that this state can be maintained up to the test instant, which instant may be essentially deferred from the ready-for-use manufacturing instant of closure device. Moreover, the circumstances prevailing during the standby period of the closure ready-for-use state may have been unfavourable to the stability of the reagent, particularly if the test kit is intended for field use.

[0006] An essential improvement capable of overcoming these problems is offered by a closure device according to the present invention having the basic construction of a sealing closure for a diagnostic test vessel or similar container, said sealing closure comprising a body part, which is tightly mountable in the mouth of the vessel and is axially made open with a cylindrical bore, said body part further including a lid for closing the bore end facing the test vessel in an openable manner, and a plunger with a diameter compatible with the bore of the body part, said plunger being slidably mounted in the bore so as to permit its movement into a sealed position with respect to the body part thus effecting the formation of a sealed reagent storage chamber in the space remaining between the lid of the body part bore end and the plunger. The essential specifications of the closure device will be evident from annexed claim 1.

[0007] The invention also concerns a method of assaying a sample, particularly a biological fluid, by way of reacting the sample in the test vessel with the assaying reagent formed by a reagent aliquot stored in the sealed closure and released from said closure into the test vessel. The method is characterized by what is stated in annexed claim 8. Furthermore, the invention concerns a test kit for clinical assay of a sample such as a blood sample. The test kit is characterized by including at least one test vessel sealed with a closure containing the reagent of the assay, said reagent having been subjected to a treatment step before the closure device is gas-tightly sealed off from communication with the ambient atmosphere.

[0008] In the following, the invention will be examined in greater detail by making reference to the appended drawings in which

Figure 1 shows a partially sectioned view of the body part of the closure device according to the invention;

Figure 2 shows a cross-sectional view of the body part of Fig. 1 in the plane A-A;

Figure 3 shows a partially sectioned view of the other basic part of the closure device according to the invention;

Figure 4 shows a partially sectioned view of the closure device according to the invention assembled into its ready-for-filling state;

Figure 5 shows a partially sectioned view of the closure device according to the invention in its ready-for-use storage state;

Figure 6 shows a partially sectioned view of the closure device according to the invention in its operat-

ing state;

Figure 7 shows the preparation step situation of an embodiment of the assay method according to the invention based on the use of the closure device according to the invention;

Figure 8 shows the calibration assay step following the step of Fig. 7 in the embodiment of the assay method according to the invention;

Figure 9 shows the use of the closure device according to the invention in the assay method; and

Figure 10 shows the actual measurement step of the assay method.

[0009] Referring to Fig. 1, the basic element of the closure device according to the invention shown therein comprises a stopper-type body part 1 shaped and dimensioned so as to fit tightly on the mouth of a vessel such as a test or reaction vessel. For tight mounting inside the mouth of the vessel, the embodiment of the body part illustrated in the diagram has annular seal ridges 6 on its skirt. Obviously, the body part may also be adapted for mounting exterior to the vessel mouth, whereby the closure device requires new shaping and dimensioning of the body part by means of conventional techniques.

[0010] To implement the structure of the closure according to the invention, the closure is provided with an axially passing cylindrical bore 2, which is best visible in the diagram of Fig. 2. A plunger 3 dimensioned to be insertable into this bore and axially movable therein with a sliding fit is shown in Fig. 3. For a tight seal between the inner surface of the closure body part bore 2 and the plunger 3, the plunger skirt is provided with a number of circular seal ridges 5 spaced apart at a distance from each other, said ridges having a special function to be described later.

[0011] In accordance with the specification of the invention, the inner wall of the centrally passing bore 2 is provided with grooves 4 running axially along the bore wall. These grooves start from that end of the body part which is oriented outward from the test vessel when the body part 1 is inserted on the mouth of the test vessel. The grooves 4 extend from said mouth of the test vessel over a certain axial length of the body part bore. The depth of the grooves 4 is made so deep as prevent the seal ridges 5 of the plunger 3 from plugging the grooves in any inserted position of the plunger in the body part bore.

[0012] An essential element of the body part 1 is a lid structure 7 made to that end of the body part which is intended to face the interior of the test vessel when the body part is mounted on the mouth of the test vessel. The function of the lid 7 is to close the vessel-side end of the bore 2 passing through the body part so as to permit an opening of the bore if so required. The opening of the lid is performed in a conventional manner by means of actuating the plunger 3 slidably adapted into the bore 2. The lid 7 is advantageously connected to the body part by means of a hinge 8, which secures the lid to the body part during the different operating states of the closure device. The inner surface of the lid 7 may include a sunken recess 9, which in its part forms a portion of the space provided inside the closure device for accommodating the test reagent to be sealed therein.

[0013] The annular seal ridges 5 of the plunger 3 are located close to the plunger end facing the interior of the test vessel. These ridges the number of which being three in the illustrated embodiment facilitate a stepwise assembly of the closure so as to maintain a gas flow communication to the ambient atmosphere from the space of the bore remaining between the closed lid 7 and the lower end of the plunger 3 facing the lid. This condition will be evident from the mutual position of the body part 1 and the plunger 3 illustrated in Fig. 4, wherein the seal ridges 5 of the plunger 3 are still positioned in the area of the grooves 4 made to the body part. When the plunger is pushed further inward, the lower ridges 5 of the plunger will reach the ungrooved wall area of the body part bore 2, thus isolating the space under the plunger from communication with the ambient atmosphere.

[0014] The assembly state of the device shown in Fig. 4, which permits gas flow communication between the reagent space 9 and the ambient atmosphere, can be utilized in the preparation of the reagent already filled into said space 9. Such a treatment may comprise, e.g., bringing the reagent into a state suitable for use in the assay and/or into a state required for the storage and handling steps of the reagent prior to the assay. Such a preparation step may include lyophilization by dry-freezing of the assay reagent and/or storage thereof under an inert gas atmosphere, sterilization of the reagent or other conventional operation which can be carried out under gas flow communication with the ambient atmosphere.

[0015] Exemplifying applications of the present closure device include assay methods based on optical measurements, in which assays the reagent must be properly dosed and prepared into a state suitable for the assay. Accurate dosing of the reagent may require charging the closure with paste-form reagent, after which the reagent must be brought into granular form for a quick assay reaction. This step can be accomplished by using above-mentioned lyophilization for moisture removal from the reagent paste.

[0016] In the assay step, the plunger inserted in the body part 1 is pushed from its initial position shown in Fig. 5 into a position shown in Fig. 6, whereby the plunger forces the lid 7 at the interior end of the body part bore 2 to snap open. Then, the reagent stored in the space 9 can fall into the test vessel in which the assay can be performed in a conventional manner.

[0017] In order to control the mutual inserted positions of the plunger 3 and the body part 1 and thus to show the operating states of the closure device, the body part is advantageously provided with a position indicator or stop 10. When the plunger 3 is pressed down into a certain position with respect to this stop, whose positions are indicated in Figs. 4, 5 and 6, the correct position of the plunger for each intended operation can thus be verified. Simultaneously, the stop acts as a protection against undesirable function, whereby the plunger and stop can be connected by means of a securing seal with each other when the closure device is in its storage, or ready-for-use, state shown in Fig. 5.

[0018] The method according to the invention is elucidated in the diagrams of Figs. 7 - 10.

[0019] In quantitative and qualitative immunological assays, generally either an antibody or antigen concentration is measured from biological fluids, excreta or tissue fluids (such as blood, sera, plasma, spinal fluid, pleural exudate, ascites, pus, wound suppuration, urine, sputum, faeces, pharyngeal smear sample, etc.). The tests may be direct, indirect or inhibitory by their nature. In immunological assays, the antibody binds to an antigen structure which is specific to said antibody. Prior to the assay, either the antibody or alternatively the antigen may be bound to a specific labelling indicator (marker). Such a marker is selected from the group of, i.a., polymeric particles (including dyed and magnetic particles), colloidal gold, stained substrates, fluorescent and phosphorescent molecules and luminescent molecules.

[0020] Quantitative assays typically utilize analyzer equipment based on optical measurement techniques (absorbance, extinction, nephelometry, reflectance, fluorescence, phosphorescence, luminescence and others). In most cases, such an optical measurement presumes elimination of error-causing optical background factors (such as lipid concentration, icterus index and other variables of the sample dependent on the status of the patient).

[0021] This background elimination is called the blank sample assay which is performed by the equipment prior to the assay of the actual analyte. After the measurement of the blank sample, the analysis equipment used in the assay starts to detect the reaction of the sample analyte with the specific reagent added to the sample solution, which is detected from a signal change chosen to be independent from other optical properties of the sample. Said signal change is selected to be proportional to the analyte concentration to be assayed in the sample.

[0022] The device and method according to the invention facilitate accurate assay of the analyte in such samples as whole blood which may have widely differing background properties.

[0023] To make the background elimination possible (using a blank sample), the reagent for the specific reaction with the analyte to be assayed is added to the sample only after the background eliminating measurement. This sequence is facilitated by the closure device according to the invention. In the method according to the invention, the reagent space 9 is filled with a specific labelling compound of an immunological test, whereby the marker may be either in the form of a free reagent (e.g., an enzyme substrate) or bound to an antibody or antigen (e.g., a substance labelled with marker particles or colloidal gold). Then, the antibody or antigen molecules can provide the required signal for the assay. Optical techniques are used to detect reagent binding or colour change, whereby kinetic measurements are possible if so required. In measurement system, the closure device according to the invention can be used as the stopper of the assay cuvette.

[0024] In a test, into an assay cuvette 11 (refer to Fig. 7) is added a required amount of buffer solution, which in the present invention is selected such that can perform a possibly required preparatory reaction (e.g., disintegration of red blood cells, known as hemolysis, or the inactivation of the Clq component of the complement of the rheumatoid factor, which is a detrimental factor in other immunological assays) in the sample to be introduced in the cuvette. After the addition of the buffer solution and the sample, the cuvette can be sealed with a device according to the invention, which acts as the closure of the cuvette, and the contents of the cuvette are stirred. Because the reagent space 9 at this stage is still separated from the sample cuvette, the labelling compound cannot mix with the solution formed by the sample and the buffer.

[0025] When required, some of the reagents such as, e.g., a hemolyzing compound (saponin) or red blood cells agglutinating compound (lectin) may be placed on the outer surface of the lid 7 in the closure device, whereby said compound can accomplish a desired preliminary reaction (hemolysis, agglutination of red blood cells) prior to the actual immunological reaction.

[0026] After the preliminary treatment (refer to Fig. 8), the sample cuvette is placed in an optically-measuring assay apparatus and the first measurement step of background elimination is carried out (on the blank sample).

[0027] After the background elimination, the passageway from the reagent space 9 of the closure device according to the invention to the interior of the sample cuvette is opened (refer to Fig. 9) by depressing the plunger of the device thus forcing the lid 7 to open. When the lid is open, the specific labelling compound is flushed from the space 9 by stirring the assembly formed by the closure device and the cuvette. Subsequent to this reagent addition step, the specific reaction of the labelling compound with the analyte can be measured by optical methods (refer to Fig. 10) without interference from the sample background.

[0028] Thence, the present invention facilitates uncomplicated storage, transfer and accurate dosing of the specific reagent at a desired instant of time. Furthermore, the invention can be utilized as a functional part

of an analytic system or assay package (test kit).

[0029] In the following, the function of the invention will be elucidated by way of examples. The examples described below are given to illustrate only a few specific applications of the above immunological assay.

Example 1

[0030] C-reactive protein (CRP) is a generally adopted indicator of an inflammation, which makes its assay from a whole-blood or serum sample of the patient a standard routine. In conjunction with CRP assay, the sample is typically analyzed using a system based on optical techniques (absorbance, extinction, nephelometry, reflectance, fluorescence, phosphorescence and others). The measurement requires a preliminary measurement on the sample (blank sample) for background elimination, whereby this step is carried out by the system prior to the assay of the actual analyte. The sample cuvette may contain different types of buffer solutions. In practice, the measurement for background elimination in CRP assay is performed by adding the whole-blood or serum sample into a sample cuvette containing a hemolytic buffer solution. Alternatively, the hemolytic reagent can be placed on the outer surface of the lid facing the sample solution. Then, the sample to be assayed may be dosed into the cuvette by means of, e.g., a capillary syringe equipped with a plunger. Next, the cuvette is closed with the closure device according to the invention serving as the stopper of the cuvette, after which the buffer solution and the sample are stirred. Subsequent to the stirring of the sample and hemolysis of red blood cells in the buffer solution, the sample cuvette is placed in the analytic apparatus. The background measurement reading of the sample is recorded and set as the zero value of the sample (blank sample).

[0031] After the background elimination of the sample, the apparatus records the reaction with the CRP of the specific reactant initiated by the release of the latter from the closure device according to the invention and subsequent mixing thereof with CRP, whereby a signal change independent from other optical properties of the sample is obtained. Thence, the signal change is made proportional to the concentration of CRP in the sample being assayed. This arrangement facilitates an accurate assay of CRP concentration in samples of widely differing background characteristics such as whole blood.

[0032] To make background elimination possible (on the blank sample), the specific reagent for CRP assay can be added only after the background elimination step. In said method, the reagent space 9 contains dry-freezed (lyophilized) polymer particles coated with CRP antibodies. As the CRP molecules will bind specifically to the antibody molecules, thus causing aggregation of the coated polymer particles, too, a dynamic measurement of the kinetic reaction by optical techniques is possible. Obviously, any other type of commonly used markers can be used (such as colloidal gold, magnetic particles, dyed particles, stained aggregates and others).

Example 2

[0033] Assay of the rheumatoid factor (RF) is extremely important in the diagnosis of different rheumatic diseases. An RF assay can be performed directly on a whole-blood or serum sample. In this test, the specific labelling particles are coated with human immunoglobulin-G molecules. In addition to the hemolyzing compound, the buffer solution of the assay reaction may contain polyanionic molecules, which bind to the Clq component of the so-called complement that otherwise could undergo a nonspecific reaction with the actual RF-labelling agent by way of binding to the Fc fragment of immunoglobulin-G. The steps of the actual test are performed in the same sequence as in Example 1. Subsequent to the addition of the blood sample, the polyanionic molecules of the assay buffer bind to the Clq component thus effectively preventing a nonspecific reaction, while the disintegration (hemolysis) of red blood cells occurs simultaneously if a whole-blood sample is being assayed. After the addition of the sample, the background elimination (using the blank sample) is performed in the same manner as in Example 1. The actual specific reaction is initiated by opening the lid 7 of the closure device according to the invention, whereby the particles coated with human immunoglobulin-G react with the RF. The aggregates formed herewith are measured in the same manner as in Example 1.

Claims

1. A closure device suitable for use in performing an assay, particularly a clinical test on a biological fluid, said device having its basic construction designed into a closure assembly suitable for closing a diagnostic test vessel or similar container, said closure device comprising a body part (1), which is suitable for tight mounting on the mouth of said vessel (1) and is provided with a cylindrical bore (2), said body part including a lid (7) suited for closing the body part bore end facing said diagnostic test vessel in an openable manner, and a plunger (3) with a diameter compatible with the bore of the body part, said plunger being slidably mounted in the bore so as to permit its movement into a sealed position with respect to the body part thus effecting the formation of a sealed reagent storage chamber (9) in the space remaining between the lid of the body part bore end and the plunger, **characterized in that** the inner wall of the bore (2) passing axially through said body part (1) is provided with at least one groove (4), whose radial depth is so deep as not to be within the reach of the outer diameter of the plunger (3), said groove extending from exterior end

of the bore, axially along the inner wall of the bore, over such a length of the bore wall as to maintain a gas flow communication between said reagent storage chamber (9) and the exterior end of the cylindrical bore when the plunger (3) is mounted into a partially inserted position.

2. A closure device as defined in claim 1, **characterized in that** said body part (1) includes a stop (10) for controlling said partially inserted position of said plunger.

3. A closure device as defined in claim 2, **characterized in that** the design of said stop (10) also permits the control of the end position of the fully inserted plunger (3).

4. A closure device as defined in any of foregoing claims 1-3, **characterized in that** the inside of said lid (7) is recessed to form the space of a reagent storage chamber (9).

5. A closure device as defined in any of foregoing claims 1-4, **characterized in that** said lid (7) is connected by a hinge (8) to said body part (1).

6. A closure device as defined in any of foregoing claims 1-5, **characterized in that** said body part (1) is detachably mountable on the mouth of a diagnostic test vessel (11) or similar container.

7. A method of performing an assay of a sample, particularly a sample of biological fluid, by way of reacting the sample with an assaying reagent formed by a reagent aliquot stored in and releasable from a closure device according to any of foregoing claims 1 - 6, sealing the test vessel, **characterized in that** said reagent is treated in its storage space in a gas flow communication therefrom with the ambient atmosphere before the storage space [closure device] is gas-tightly sealed off from communication with the ambient atmosphere.

8. A method as defined in claim 7, **characterized in that** said assay reagent is [a] lyophilized [reagent] in the storage space of the closure device.

9. A method as defined in claim 7, **characterized in that** said assay reagent is [a reagent stored] protected by an inert gas atmosphere.

10. A test kit particularly for performing a clinical assay of a sample such as a blood sample, **characterized in that** said kit includes at least one test vessel sealed with a closure according to claim 1, containing the reagent of the assay, said reagent having been subjected to a treatment step before the closure device is gas-tightly sealed off from communication with the ambient atmosphere.

Patentansprüche

1. Verschlußvorrichtung, die zur Verwendung bei der Durchführung einer Analyse bzw. eines Assays, insbesondere eines klinischen Tests an einem biologischen Fluid, geeignet ist, wobei die Vorrichtung in ihrer Grundkonstruktion als eine Verschlußanordnung ausgeführt ist, die geeignet ist, ein Diagnosetestgefäß oder einen ähnlichen Behälter zu verschließen, wobei die Verschlußvorrichtung aufweist:

   einen Körperabschnitt (1), welcher geeignet ist, um dicht am Mund des Gefäßes (1) angebracht zu werden, und welcher mit einer zylindrischen Bohrung (2) versehen ist, wobei der Körperabschnitt einen Deckel (7) aufweist, der geeignet ist, um das zum Diagnosetestgefäß liegende Ende der Körperabschnitt-Bohrung auf wieder zu öffnende Weise zu verschließen, und
   einen Kolben (3) mit einem zur Bohrung des Körperabschnitts passenden Durchmesser, wobei der Kolben gleitbar in der Bohrung angeordnet ist, um so seine Bewegung in eine Abdichtungsposition in bezug auf den Körperabschnitt zu erlauben, wodurch sich im verbleibenden Raum zwischen dem Deckel des Endes der Körperabschnitt-Bohrung und dem Kolben eine dichte Reagensaufbewahrungskammer (9) bildet,

   dadurch gekennzeichnet, daß die Innenwand der axial durch den Körperteil (1) verlaufenden Bohrung (2) mit mindestens einer Rille (4) versehen ist, deren radiale Tiefe so tief ist, daß sie nicht in Reichweite des Außendurchmessers des Kolbens (3) ist, wobei sich die Rille von dem äußeren Ende der Bohrung axial entlang der Innenwand der Bohrung über eine solche Länge erstreckt, um, wenn der Kolben (3) in einer teilweise eingeschobenen Position eingesetzt ist, eine Gasstromverbindung zwischen der Reagensaufbewahrungskammer (9) und dem äußeren Ende der zylindrischen Bohrung aufrechtzuerhalten.

2. Verschlußvorrichtung nach Anspruch 1, **dadurch gekennzeichnet, daß** der Körperabschnitt (1) einen Anschlag (10) zur Steuerung der teilweise eingeschobenen Position des Kolbens aufweist.

3. Verschlußvorrichtung nach Anspruch 2, **dadurch gekennzeichnet, daß** die Gestaltung des Anschlags (10) auch die Steuerung der Endposition des voll eingeschobenen Kolbens (3) erlaubt.

4. Verschlußvorrichtung nach einem der vorstehenden Ansprüche 1 bis 3, **dadurch gekennzeichnet, daß** das Innere des Deckels (7) ausgespart ist, um den Raum einer Reagensaufbewahrungskammer (9) zu bilden.

5. Verschlußvorrichtung nach einem der vorstehenden Ansprüche 1 bis 4, **dadurch gekennzeichnet, daß** der Deckel (7) über ein Scharniergelenk (8) mit dem Körperabschnitt (1) verbunden ist.

6. Verschlußvorrichtung nach einem der vorstehenden Ansprüche 1 bis 5, **dadurch gekennzeichnet, daß** der Körperabschnitt (1) lösbar am Mund eines Diagnosetestgefäßes (11) oder eines ähnlichen Behälters anbringbar ist.

7. Verfahren zur Durchführung einer Analyse einer Probe, insbesondere einer Probe eines biologischen Fluids, mittels Reaktion der Probe mit einem Nachweisreagens, das von einem Reagensaliquot gebildet wird, das in der Verschlußvorrichtung gemäß einem der vorstehenden Ansprüche 1 bis 6, die das Testgefäß verschließt, aufbewahrt und daraus freisetzbar ist, **dadurch gekennzeichnet, daß** das Reagens in seinem Aufbewahrungsraum, der in einer Gasstromverbindung mit der Umgebungsatmosphäre steht, behandelt wird, bevor der Aufbewahrungsraum gasdicht von der Verbindung zur Umgebungsatmosphäre abgedichtet wird.

8. Verfahren nach Anspruch 7, **dadurch gekennzeichnet, daß** das Analysereagens im Aufbewahrungsraum der Verschlußvorrichtung lyophilisiert wird.

9. Verfahren nach Anspruch 7, **dadurch gekennzeichnet, daß** das Analysereagens [ein] durch eine inerte Gasatmosphäre geschützt [aufbewahrtes Reagens] ist.

10. Testkit insbesondere zur Durchführung einer klinischen Analyse einer Probe wie beispielsweise einer Blutprobe, **dadurch gekennzeichnet, daß** das Kit mindestens ein Testgefäß aufweist, das mit einem das Reagens der Analyse enthaltenden Verschluß nach Anspruch 1 abgedichtet wird, wobei das Reagens einem Behandlungsschritt unterzogen worden ist, bevor die Verschlußvorrichtung von einer Verbindung zur Umgebungsatmosphäre gasdicht abgedichtet worden ist.

Revendications

1. Bouchon convenant pour une utilisation dans l'exécution d'une analyse, en particulier d'un test clinique sur un fluide biologique, la construction de base du dit bouchon étant sous la forme d'un dispositif de fermeture convenant pour fermer un récipient de test de diagnostic ou un récipient similaire, le dit bouchon comprenant un corps (1), qui est prévu pour un montage serré sur l'embouchure du dit récipient (1) et qui comporte un passage intérieur cylindrique (2), le dit corps incluant un couvercle (7) qui peut fermer l'extrémité du passage intérieur du corps en regard du dit récipient de test de diagnostic et qui peut être ouvert, et un plongeur (3) ayant un diamètre compatible avec le passage intérieur du corps, le dit plongeur étant monté de façon coulissante dans le passage afin de permettre son mouvement d'amenée à une position d'étanchéité par rapport au corps de manière à définir une chambre fermée de stockage de réactif (9) dans l'espace restant entre le couvercle de fermeture de l'extrémité du passage du corps et le plongeur, **caractérisé en ce que** la paroi intérieure du passage (2) qui traverse axialement le dit corps (1) comporte au moins une rainure (4) dont la profondeur radiale est assez grande pour ne pas se trouver dans la limite du diamètre extérieur du plongeur (3), la dite rainure s'étendant à partir de l'extrémité extérieure du passage, axialement le long de la paroi intérieure du passage, sur une longueur de la paroi du passage telle qu'une communication d'écoulement de gaz est maintenue entre la dite chambre de stockage de réactif (9) et l'extrémité extérieure du passage cylindrique lorsque le plongeur (3) est monté dans une position d'insertion partielle.

2. Bouchon selon la revendication 1, **caractérisé en ce que** le dit corps (1) comporte une butée (10) pour déterminer la dite position d'insertion partielle du dit plongeur.

3. Bouchon selon la revendication 2, **caractérisé en ce que** le dessin de la dite butée (10) permet également de déterminer la position d'extrémité du plongeur complètement inséré (3).

4. Bouchon selon une quelconque des revendications précédentes 1 à 3, **caractérisé en ce que** l'intérieur du dit couvercle (7) est évidé pour former l'espace d'une chambre de stockage de réactif (9).

5. Bouchon selon une quelconque des revendications précédentes 1 à 4, **caractérisé en ce que** le dit couvercle (7) est relié au dit corps (1) par une charnière (8).

6. Bouchon selon une quelconque des revendications précédentes 1 à 5, **caractérisé en ce que** le dit corps (1) peut être monté de façon séparable sur l'embouchure d'un récipient de test de diagnostic (11) ou d'un récipient similaire.

7. Procédé d'exécution d'une analyse d'un échantillon, en particulier un échantillon de fluide biologique, par réaction de l'échantillon avec un réactif d'analyse formé par une partie aliquote de réactif stockée dans un bouchon et qui peut être extraite de ce bouchon selon une quelconque des revendications précédentes 1 à 6, fermant le récipient de test, **caractérisé en ce que** le dit réactif est traité dans son espace de stockage, en communication d'écoulement de gaz de cet espace avec l'atmosphère ambiante, avant que l'espace de stockage soit isolé de façon étanche aux gaz de la communication avec l'atmosphère ambiante.

8. Procédé selon la revendication 7, **caractérisé en ce que** le dit réactif d'analyse est lyophilisé dans l'espace de stockage du bouchon.

9. Procédé selon la revendication 7, **caractérisé en ce que** le dit réactif d'analyse est [un réactif stocké] protégé par une atmosphère de gaz inerte.

10. Trousse de test, en particulier pour effectuer une analyse clinique d'un échantillon tel qu'un échantillon de sang, **caractérisée en ce que** la dite trousse comprend au moins un récipient de test fermé par un bouchon selon la revendication 1, contenant le réactif de l'analyse, le dit réactif ayant été soumis à une étape de traitement avant que le bouchon soit isolé de façon étanche aux gaz d'une communication avec l'atmosphère ambiante.

Fig. 1

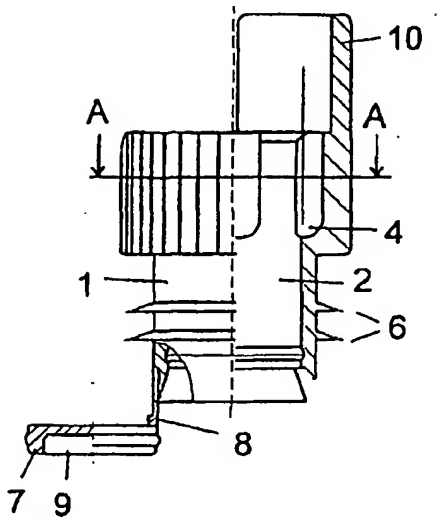

Fig. 2

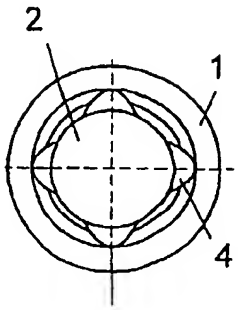

Fig. 3

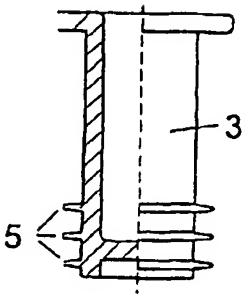

Fig. 4

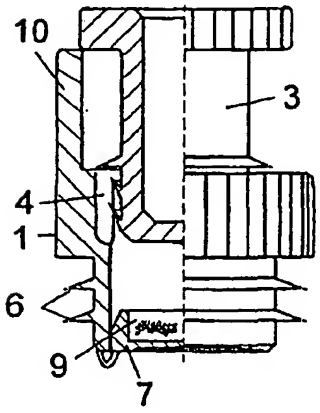

Fig. 5

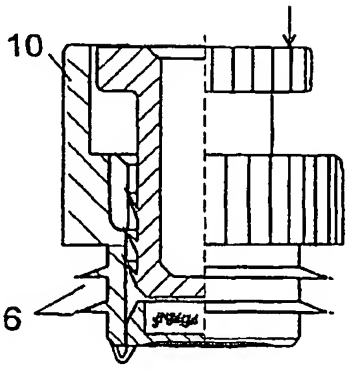

Fig. 6

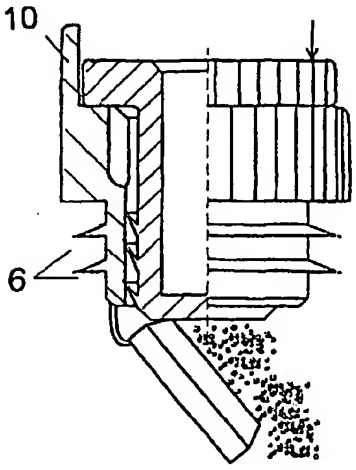

Fig. 7

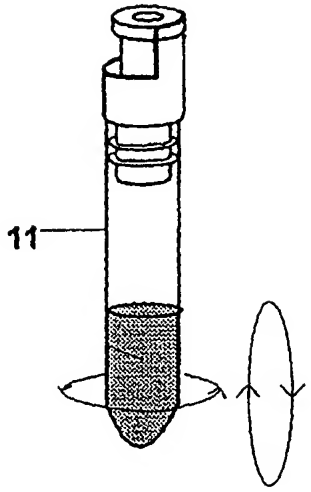

Fig. 8

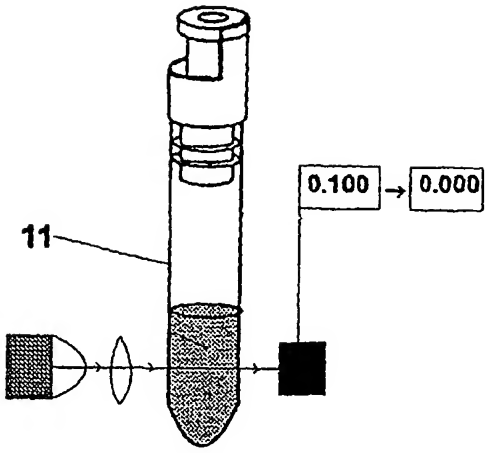

Fig. 9

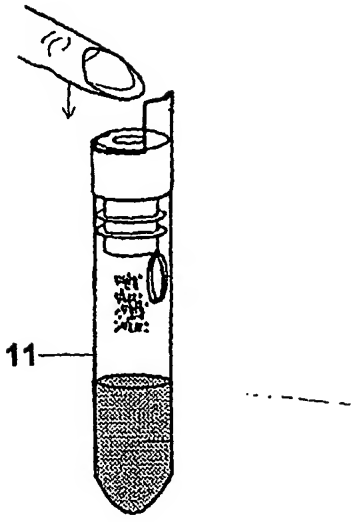

Fig. 10